miss. Recent data from our experience with the active CPT-11/oxaliplatin combination will be presented, as well as published or ongoing results of CPT-11/5-FU combinations and oxaliplatin/5-FU combinations. We are now in colorectal cancer treatment exactly where we were twenty years ago with breast cancer. We should not repeat time wasting errors, and try to take the opportunities without waiting for metanalysis or consensus decisions making.

1297

Adjuvant therapy: how effective, and for which patients? A meta- analysis

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Chemotherapy can improve survival in colorectal cancer. To help define the size of benefit achievable for different types of patient, and the optimal chemotherapy regimen, a meta-analysis of all randomised trials comparing chemotherapy with surgery alone was undertaken. Individual patient data from a systematic overview of studies starting before 1987, was supplemented by published data from more recent studies. Almost all chemotherapy regimens tested involved 5-fluorouracil (5-FU), with or without other drugs. The 50 studies, involving 18,000 patients, were divided into broad groups based on pharmacokinetic considerations. As anticipated, short bolus chemotherapy regimens appeared the least effective. But, when 5-FU was given as a one-week continuous infusion through the portal vein the annual death rate was reduced by 14%SD5 ($p = 0.006$). Considering all prolonged systemic chemotherapy regimens together, the death rate was reduced by 11%SD3 ($p = 0.001$). However, the benefits seen in studies of 5-FU biomodulated by folinic acid (29%SD9: $p = 0.0007$) or by levamisole (22%SD9; $p = 0.01$) were significantly larger than in studies testing unmodulated 5-FU regimens (6%SD4; $p = 0.11$). There remain unanswered questions about who should be treated as most trials of 5-FU/folinic acid included only colon cancer patients and most of the benefit seen in them was among Dukes stage C (N+) patients. It seems reasonable to extrapolate from colon to rectal cancer as in the earlier trials of unmodulated 5-FU the benefits appeared similar for rectal and colon cancer. But, for stage B (N−) patients worthwhile benefit is not yet firmly established and more randomised evidence is needed.

1298

Laparoscopic surgery for colorectal cancer – Is it safe?

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Laparoscopic surgery for colorectal cancer is one of the most controversial applications of the new wave of endoscopic procedures for digestive diseases. The principal concerns revolve around the adequacy of margins of excision and the development of unusual patterns of recurrence such as port site recurrences. Despite the publication of large individual series of laparoscopic resections for colorectal cancer the issues surrounding the health care economics and patterns of recurrence have not yet been resolved. Port site recurrences have been reported to occur more frequently than do wound recurrences with conventional open surgery, but in the author's experience of over eighty laparoscopic colorectal resections there has not yet been one port site recurrence.

However, in both the authors' experience and that of others data are emerging to suggest that hospital stay is not significantly diminished by the use of laparoscopic surgery alone and other factors such as early restoration of nutrition, anaesthetic management and forced mobilisation may be more important, but if so are equally applicable to those undergoing conventional open surgery. None of these debates will be resolved by the publication of further series by individual surgeons of groups and the results of randomised clinical trials must be awaited. In the UK, the Medical Research Council's CLASICC (Conventional versus Laparoscopic-Assisted Surgery in Colorectal Cancer) trial is now under way and is recruiting rapidly. The trial incorporates both pathological surrogate end points as well as clinical ones and has major quality of life and health care economic studies embedded in it. It is anticipated that this, and similar trials, will finally allow a decision to be made as to whether laparoscopic surgery for colorectal cancer is safe and cost effective.

1299

Tumour specific antigens: Perspectives for vaccination

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Cytolytic T lymphocytes (CTL) that specifically lyse autologous tumour cells are often found in the blood of cancer patients. Many of the tumor antigens recognized by such CTL have been identified over the last six years. Several of them are truely tumour specific antigens and they are now be used to try to induce or to enhance tumour rejection responses in cancer patients.

There are three main classes of tumour antigens recognized by CTL: antigens encoded by genes, such as the MAGE genes, that are expressed in many tumors but that are silent in most normal tissues; differentiation antigens, such as tyrosinase, that are only expressed in normal melanocytes and in melanomas; and antigens encoded by genes that are mutated in the tumour cells. Although it seems very likely that many other tumor antigens are still to be identified, the priority is now to demonstrate that immunization against some of these antigens is clinically valuable.

A small number of patients with advanced disease received several injections of an antigenic peptide encoded by gene MAGE-3, in the absence of adjuvant. Tumour regressions were observed in 5 out of 17 melanoma tumour-bearing patients. These preliminary results might be improved by testing other modalities of immunization such as peptides or proteins combined with adjuvants, recombinant adenoviruses or poxviruses containing the genes encoding the antigens, or antigen presenting cells such as dendritic cells, incubated *in vitro* with the antigens and injected back into the patient.

1300

Vaccination against lung cancer: Animal models

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Cytolytic T lymphocytes (CTL) directed against peptides presented by MHC class I molecules, constitute powerful effectors of the immune system against tumors. CTL recognizable peptide antigens have been isolated from human and murine tumors. We have isolated two Tumor Associated Antigen (TAA) peptides from a murine metastatic lung carcinoma (3LL-D122). One of the peptides, derived from a mutated connexin 37 gene (MUT1) constitutes a shared TAA between two independent lung carcinomas. Peptide vaccines based on MUT1 can cause rejection of established D122 micrometastases when the peptides are loaded on effective Antigen Presenting Cells (APCs) like the TAP deficient RMA-S cells. Syngeneic fibroblasts (BLK cells) and IL-6 transduced BLK cells loaded with MUT1 can also serve for vaccination while IL-2 transduced BLK vaccines were found to have reduced efficacy.

A second TAA peptide, He-9, was shown to be derived from an aberrant β-globin gene expressed in 3LL lines. The ability of the peptides and other K^b binding β-globin peptides to induce anti-tumor CTL versus autoimmune effects will be described.

1301

Preclinical and clinical experience with peptide-based vaccines against HPV16-induced tumors

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T-cell immunity occurs naturally against tumors induced by viruses and other causes. In the latter case self antigens are increasingly found to be targets of tumor associated CTL. In all categories of tumors the T cell response usually falls short of the maximally possible response. This situation calls for vaccination, primarily in situations of low tumor burden and adoptive transfer with tumor specific T cells in case of higher tumor burden. Indeed we recently observed that patients with HPV16 positive cervical carcinomas or CIN lesions only rarely show CTL responses against predicted HPV16 epitopes presented by HLA class I molecules.

In a mouse HPV16 positive tumor model we found that effective protection against $HPV16^+$ tumor inocula could be achieved by vaccination with an HPV 16 E7 derived peptide in incomplete Freund adjuvant (IFA) or pulsed onto dendritic cells (DC) or with E7 protein in IFA or pulsed onto DC. In a clinical HPV16 vaccination trial 15 patients have been vaccinated with either of three escalating doses of two HLA-A*0201 binding CTL-inducing peptides and a helper peptide binding to all known HLA-DR molecules, mixed with Montanide ISA 51 adjuvant. No toxicity was associated with

vaccine delivery at any dose. Two patients vaccinated with the lowest vaccine dose showed stable disease during more than a year. The patients with the intermediate and highest vaccine doses are still monitored with respect to clinical response. The results of CTL and T helper response assays against the individual vaccine components will be presented.

1302

Immune responses to peptide-based cancer vaccines

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Three classes of antigens recognized by cytotoxic T lymphocytes (CTL) are defined in melanoma and some other tumors: 1. Cancer-testis (CT) antigens (MAGE, BAGE, GAGE), expressed in tumors and testis, 2. Differentiation antigens (Melan A/MART-1, tyrosinase gp100/Pmel17, gp75), expressed in melanoma and melanocytes 3. Antigens defined by mutations (CDK-2/R24C, MUM-1, β-catenin). Target structures for CTL recognition are peptides of 9–10 aminoacids length. These peptides bind to MHC class I molecules and are presented at the tumor cell surface. Synthetic peptides can generate specific CTL in vitro that effectively lyse tumor cells expressing the corresponding antigen. Clinical trials using MAGE-1 and MAGE-3 peptides for immunization in HLA-A1+ patients with MAGE-expressing tumors showed objective responses (CR/PR) in some melanoma patients. We immunized HLA-A2+ melanoma patients with peptides derived from Melan A/MART-1, tyrosinase, and gp100/Pmel17. Delayed-type-hypersensitivity reactions (DTH) and peptide-specific CTL responses as well as objective tumor regressions were observed in 3/12 patients. Subsequently, the effects of systemic GM-CSF on immune reactions to peptide vaccines were assessed. Enhanced DTH reactions were observed with infiltrates of CD4+ and CD8+ T lymphocytes, CD1a+ Langerhans cells, and a strong expression of IL-2 and γIFN, suggesting the activation of CD4+ Th1 and CD8+ CTL. Objective tumor regressions were documented in 5/16 patients. The identification of further tumor associated antigens recognized by CTL and the use of adjuvants to enhance their immunogenicity will open broad perspectives for the development of polyvalent cancer vaccines to control or inhibit tumor growth in vivo and to prevent tumor escape of antigen-loss variants.

1303

Active immunization of melanoma patients with IL-2- OR IL-4-transduced allogeneic melanoma cells

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The aim of these clinical studies was to immunize stage IV melanoma patients with HLA-A2-compatible, immunogeneic human melanoma lines genetically modified to release IL-2 or IL-4 in order to elicit or increase a T cell-mediated anti-melanoma response which may affect distant melanoma lesions. These lines were characterized for transgene expression and for the presence of immunological relevant molecules before in vivo vaccination. Twelve patients were treated with IL-2 releasing line while 10 patients were treated with IL-4 gene transduced melanoma cells. The side effects of the treatment were locally mild and systemically absent. All patients were assessable for clinical response and received at least 3 vaccine administrations. Three and 2 mixed responses were clinically observed in group of patients treated with IL-2 and IL-4 transduced melanoma lines, respectively. To evaluate specific immune response, limiting dilution experiments and mixed tumor-lymphocytes cultures were performed using different HLA-A2 compatible melanoma lines, autologous line when available and peptides obtained from known melanoma antigens. This immunological monitoring was performed on peripheral blood lymphocytes obtained from each patient before and after vaccination. An increased frequency of lytic and specific lymphocyte precursors was observed in some cases. Histological and immunohistochemical analyses of biopsies obtained before and after vaccination from tumor nodules and sites of vaccine injection are in progress and will be presented.

1304

Mutant ras peptide vaccines

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ras proto-oncogenes activated by point mutations within codon 12, 13 or 61 are frequently found in human tumours. Since mutant p21 ras molecules encoded by these oncogenes are specific for cancer cells, mutant p21 ras or ras-derived peptides are attractive candidates for a cancer vaccine.

We have studied T cell responses to mutant p21 ras in both healthy volunteers and in cancer patients for the purpose of developing therapeutic and prophylactic cancer vaccines. From these studies we were able to define optimal peptides for T cell stimulation that contain overlapping epitopes capable of stimulating both CD4 and CD8 T cell responses. Studies with a large number of T cell clones derived from different donors, including cancer patients demonstrated that binding of ras derived peptides to HLA class II molecules is promiscuous. Together these results indicated that immunotherapy targeted against neoplastic cells carrying ras mutations is possible.

We have now completed a pilot phase I/II clinical study, and in some of the patients we were able to elicit an immune response by vaccination with autologous ras-peptide loaded antigen presenting cells. The responding cells were both of the CD4 Th1 and CD8 phenotype and were able to kill autologous tumor cells as well as other tumor cells carrying the same ras mutation (12Gly→Val) These results indicate that ras peptide vaccination may result in the generation of a potentially beneficial immune response in cancer patients. We are presently conducting several new clinical protocols based on ras peptide vaccination of patients with different forms of cancer, either by direct intradermal injection of peptides and using recombinant human GM-CSF as adjuvant, or by intralymphatic injection of peptide pulsed dendritic cells.

1305

Oncogene activation as a prognostic marker in head and neck cancer

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In cancer numerous chromosomal abnormalities (e.g. deletions, amplifications and translocations) are associated with the (in-)activation of tumorsuppressor and oncogenes. The detection of genetic aberrations has clinical relevance in classifying tumors or as prognostic factor. For instance, carcinomas of the head/neck region (HNSCC) frequently show amplification of EGFR and the chromosome 11q13 region, point-mutations in p53 and loss of $p16^{INK4}$. We have focused on DNA amplification of the chromosome 11q13 region that was found in 36% of HNSCC. (1) Comparison of HNSCC-patients with and without DNA amplification revealed that amplification is correlated with poor prognosis. (2) We have identified two genes *cyclin D1* and *EMS1/cortactin* that are overexpressed due to DNA amplification. For a proper understanding of the biological behavior of tumors with 11q13 amplification, we introduced these genes into cells *in vitro* to study the effect on (cell) biological properties. (3) With antibodies against the gene products, we have developed an immunohistochemical screening method that is evaluated by comparisons to southern blot and interphase FISH data. Reliable and easier detection methods will enable us to screen large series of HNSCC, to refine the classification of relevant tumors, and ultimately to design more rational therapies. All these aspects will be discussed.

1306

Predicting response in head and neck cancer: The search for the Holy Grail

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A standard radiation therapy schedule is not the optimal treatment for all patients presenting with head and neck (H&N) cancer. To modify the treatment parameters, predictive tests are required allowing to discriminate subpopulations of patients for whom the modification of treatment parameters could be beneficial. We intend to review the current status on predictive tests especially aimed at determining proliferation status (PS),